CCLVII.—*The Constitution of Ergothioneine: a Betaine Related to Histidine.*

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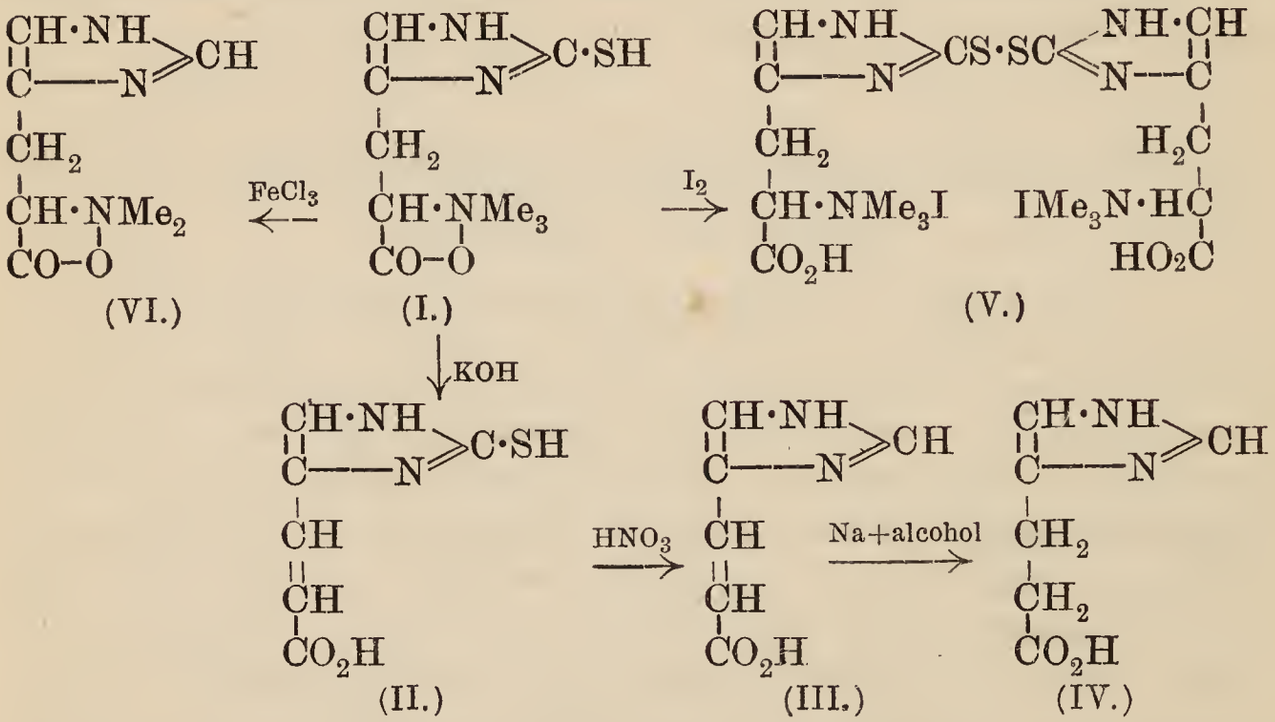
Two years ago Tanret (*J. Pharm. Chim.*, 1909, [vi], **30**, 145) isolated from ergot a new crystalline base containing sulphur, and having the formula $C_9H_{15}O_2N_3S$. The description of the base given by Tanret suggested to us a possible relationship to the amino-acid histidine; thus ergothioneine, as the new base was called by Tanret, is precipitated by mercuric chloride, and forms a compound with silver. These properties, together with the large nitrogen content, were strongly suggestive of the presence of a glyoxaline ring. Since one of us in conjunction with H. H. Dale (Trans., 1910, **97**, 2592) had already isolated another histidine derivative from ergot, namely, 4(or 5)-β-aminoethylglyoxaline, a substance of great physiological activity and interest, we prepared a small quantity of ergothioneine according to Tanret's method, and found that, like histidine and many other glyoxaline derivatives, it gives an intense red coloration with sodium *p*-diazobenzenesulphonate, thus still further supporting our surmise with regard to the constitution of this base.

Ergothioneine contains (in addition to the sulphur atom) three carbon atoms and six hydrogen atoms more than histidine. These might well result from the substitution of three hydrogen atoms of histidine by methyl groups in such a manner that ergothioneine would be a new member of the class of methylated amino-acids or betaines, of which a number of examples are now known to occur in plants. This hypothesis proved to be correct; the substance is indeed a betaine, and almost certainly β-2-thiolglyoxaline-4(or 5)-propiobetaine (I). Like other betaines it has no marked physiological action.

On boiling with a concentrated (50 per cent.) aqueous solution of potassium hydroxide, the base is decomposed quantitatively into

trimethylamine and a yellow acid of the composition $C_6H_6O_2N_2S$, which represents the whole of the remaining atoms in the molecule, and the constitution of which is represented by (II). On boiling with dilute nitric acid, the sulphur atom of this acid is completely removed, and a new acid, β-glyoxaline-4(or 5)-acrylic acid (III), is formed. This acid on reduction yields β-glyoxaline-4(or 5)-propionic acid (IV), and we were able to identify both the saturated and the unsaturated acid by comparison with synthetic specimens. The various reactions will be best seen from the following scheme:



The only doubtful point remaining was with regard to the position of the sulphur atom. There are two probable positions, namely, attachment to the β-carbon atom of the side-chain or to the carbon atom in the 2-position in the glyoxaline ring, and choice between these two, although decisive, is based on analogy rather than on direct evidence; indeed, synthesis seems to be the only means of supplying complete proof.

The sulphur in ergothioneine reacts in every way like that of the thiolglyoxalines, and quite different from that in cystein, with which it might be expected to show analogies if attached to the β-carbon atom of the side-chain; thus the sulphur in cystein, as is well known, is readily eliminated by boiling with sodium hydroxide, whereas, as was stated above, the sulphur of ergothioneine is not removed by boiling with the strongest solutions of potassium hydroxide. On the other hand, the sulphur atom, like that of the thiolglyoxalines, is readily and quantitatively oxidised by ferric chloride (as recently employed by Pyman) or by bromine water to sulphuric acid, whereas in the case of cystein a sulphonic acid results, in which the sulphur still remains attached to the carbon atom. The only oxidising agent which acts similarly on cystein and ergo-

thioneine is iodine, which oxidises both to a compound, in which two sulphur atoms are directly linked. That obtained from ergothioneine has the constitution (V).* A further argument for considering ergothioneine to be a thiolglyoxaline derivative is the fact that, like other betaines, it is a feeble mono-acid base, whereas we should expect it to be di-acid if it, like histidine, contained the simple glyoxaline ring. The basic properties of the ring are destroyed by the presence of the sulphur atom as in other thiolglyoxalines (compare, for instance, 2-thiol-4(or 5)-aminomethylglyoxaline, which forms a mono-hydrochloride, and was recently described by Pyman, Trans., 1911, **99**, 672).†

Treatment of ergothioneine with ferric chloride gave trimethylhistidine (VI), a betaine which may possibly occur in nature.

Experimental.

The Action of Boiling 50 *per cent. Aqueous Potassium Hydroxide on Ergothioneine.*

0·88 Gram of ergothioneine was boiled with 20 c.c. of a 50 per cent. aqueous solution of potassium hydroxide, the distillate being collected in a known volume of *N*-hydrochloric acid. Distillation was continued until the evolution of alkaline vapours was practically complete. It was found that 3·1 c.c. of *N*-hydrochloric acid had been neutralised, corresponding with 80 per cent. of the theoretical for the evolution of one nitrogen atom. The main bulk of the distillate was evaporated to dryness, and the residue dissolved in a little alcohol and treated with an alcoholic solution of platinic chloride, when trimethylamine platinichloride (m. p. 241°) separated. (Found, Pt = 36·9. Calc., Pt = 36·9 per cent.)

One nitrogen atom was thus shown to be evolved as trimethylamine. The strongly alkaline residue in the flask was rendered acid to Congo-red, when there was at once precipitated as a yellow, amorphous solid, *β-2-thiolglyoxaline-4-acrylic acid.*

This acid was found to be characterised by its general insolubility.

* This iodide will be described later. It was already obtained by Tanret, who did not, however, appreciate its true significance. It forms black, steel grey or blue mixed crystals with iodine, a peculiar and extremely rare property which has so far only been observed in the case of cholalic acid, narceine, and saponarin, and is closely analogous to the adsorption of iodine by starch. For this reason Tanret, who analysed the compound, did not obtain results in agreement with any simple formula.

† Dr. Pyman has since informed us of his recent observation that thiolglyoxalines are sharply differentiated from glyoxalines by the fact that they at once decolorise a dilute cold solution of potassium permanganate; we find that ergothioneine also does this, but histidine does not, nor does cystine.

It is only moderately soluble in pyridine, and almost insoluble in all other ordinary organic solvents. The acid was crystallised for analysis by making a very dilute solution (0·1 per cent.) of the sodium salt, and acidifying. After three or four hours the acid began to separate, and precipitation was complete in about thirty-six hours. The acid obtained in this way separated in clusters of small prisms, which did not melt below 275°:

0·1179 gave 0·1805 CO_2 and 0·0420 H_2O. C = 41·8; H = 3·9.
$C_6H_5O_2N_2S$ requires C = 42·3; H = 3·5 per cent.

By oxidation by means of dilute nitric acid as described below, 90 per cent. of the theoretical amount of sulphur was obtained from the filtrate as barium sulphate.

The Action of Boiling Dilute Nitric Acid on β-2-Thiolglyoxaline-4-acrylic Acid.

0·4 Gram of amorphous β-2-thiolglyoxaline-4-acrylic acid was added in small portions to 20 c.c. of 10 per cent. aqueous nitric acid. The mixture was kept gently boiling on a sand-bath for some minutes after solution was complete, and then allowed to cool. The crystalline *nitrate* of *β-glyoxaline-4(or 5)-acrylic acid* separated out, which after washing and drying melted at 198° with explosive decomposition. The yield was 0·28 gram:

0·1114 gave 0·1470 CO_2 and 0·0404 H_2O. C = 36·0; H = 4·0.
$C_6H_6O_2N_2,HNO_3$ requires C = 35·8; H = 3·5 per cent.

β-Glyoxaline-4(or 5)-acrylic acid was most readily obtained from the nitrate by the addition of one equivalent of sodium carbonate (solid) to a concentrated aqueous solution of the salt, when the acid separates at once as a crystalline solid, which after recrystallisation from dilute acetone was quite pure, and melted at 235—236°:

0·1776 gave 0·3474 CO_2 and 0·0712 H_2O. C = 53·3; H = 4·4.
$C_6H_6N_2O_2$ requires C = 52·2; H = 4·3 per cent.

β-Glyoxaline-4(or 5)-acrylic acid is moderately soluble in cold, but very readily so in hot, water. Its solutions give an intense red colour with sodium *p*-diazobenzenesulphonate.

The *phosphotungstate* is readily soluble in hot water or cold acetone, and crystallises in small, rectangular plates from dilute acetone.

The *picrate* forms golden-yellow prisms, melting at 213—214°, and sparingly soluble in cold water. The melting point of this picrate

remained unchanged when mixed with a synthetic specimen. The latter we obtained in small quantity by the action of trimethylamine on α-chloro-β-glyoxaline-4(or 5)-propionic acid.

The Reduction of β-Glyoxaline-4(or 5)-acrylic Acid to β-Glyoxaline-4(or 5)-propionic Acid.

β-Glyoxaline-4(or 5)-acrylic acid was dissolved in a little absolute alcohol, and the hot solution treated with five to six atomic proportions of sodium. The solution was then acidified with hydrochloric acid, and the precipitated salt collected. The filtrate and washings were evaporated to dryness, the residue dissolved in 5 per cent. aqueous sulphuric acid, and precipitated with phosphotungstic acid. The precipitate was treated with acetone, filtered from undissolved material, and the soluble phosphotungstate decomposed in the usual manner. On concentrating the final filtrate a crystalline acid separated, which after recrystallisation melted at 202°. When mixed with a specimen of synthetic β-glyoxaline-4(or 5)-propionic acid (m. p. 202°), the melting point remained unaltered. The acid obtained on reduction was therefore proved to be β-glyoxaline-4(or 5)-propionic acid.

The Action of Ferric Chloride on Ergothioneine. Formation of β-Glyoxaline-4(or 5)-propiobetaine (Histidine-betaine).

One gram of ergothioneine was boiled for one hour with an aqueous solution containing nine molecular proportions of ferric chloride. The iron was removed from solution by sodium carbonate, the filtrate acidified with sulphuric acid (so as to give a 5 per cent. solution of the latter), and precipitated by phosphotungstic acid. The precipitate, which was completely soluble in acetone, was decomposed in the usual way, and the barium and sulphuric acid removed. The filtrate was concentrated, and treated with a hot aqueous solution of picric acid. On cooling, a crystalline picrate separated, which after recrystallisation from water was obtained in deep yellow prisms melting at 123°:

0·1340 gave 0·1908 CO_2 and 0·0396 H_2O. C = 38·4; H = 3·3.

$C_9H_{15}O_2N_3(C_6H_3O_7N_3)_2$ requires C = 38·5; H = 3·3 per cent.

The *dipicrate* is very sparingly soluble in cold, fairly readily so in hot, water.

The *picrolonate* prepared in the usual way forms long, thin, orange-yellow needles, melting at 229—230°.

The *aurichloride* separated from dilute aqueous hydrochloric acid as large, broad, deep orange-yellow prisms, melting at 171°.

In conclusion, we wish to express our indebtedness to Dr. F. L. Pyman for synthetic specimens of α-chloro-β-glyoxaline-4(or 5)-propionic and β-glyoxaline-4(or 5)-propionic acids for purposes of comparison, and to the Wellcome Chemical Works, Dartford, for a supply of ergothioneine.

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